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Ingestion of insoluble dietary fibre increased zinc and iron absorption and restored growth rate and zinc absorption suppressed by dietary phytate in rats

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We examined the effects of ingestion of five types of insoluble fibre on growth and Zn absorption in rats fed a marginally Zn-deficient diet (6·75 mg (0·103 mmol) Zn/kg diet) with or without added sodium phytate (12·6 mmol/kg diet). The types of insoluble fibre tested were corn husks, watermelon skin, yam-bean root (*Pachyrhizus erosus*) and pineapple core, and cellulose was used as a control (100 g/kg diet). Body-weight gain in the cellulose groups was suppressed by 57 % by feeding phytate. Body-weight gain in phytate-fed rats was 80 % greater in the watermelon skin fibre and yam-bean root fibre group than that in the cellulose group. Zn absorption ratio in the cellulose groups was lowered by 46 and 70 % in the first (days 7–10) and second (days 16–19) measurement periods with feeding phytate. In the rats fed the phytate-containing diets, Zn absorption ratio in the watermelon skin, yam-bean root and pineapple core fibre groups was 140, 80 and 54 % higher respectively than that in the cellulose group, in the second period. Fe absorption was not suppressed by phytate, however, feeding of these three types of fibre promoted Fe absorption in rats fed phytate-free diets. The concentration of soluble Zn in the caecal contents in the watermelon skin fibre or yam-bean root fibre groups was identical to that in the control group in spite of a higher short-chain fatty acid concentration and lower pH in the caecum. These findings indicate that ingestion of these types of insoluble fibre recovered the growth and Zn absorption suppressed by feeding a high level of phytate, and factors other than caecal fermentation may also be involved in this effect of insoluble fibre.

Insoluble dietary fibre: Zinc absorption: Phytic acid: Caecal fermentation: Bulking effect: Rats

Zn is an essential element required for function of insulin, neuropeptides and many enzymes such as DNA polymerase and RNA polymerase. Lowering of gustatory and olfactory sensitivity and immunological defects are major signs of Zn deficiency (Cousins, 1996). Severe Zn deficiency causes growth repression and anorexia (Giugliano & Millward, 1984).

Some food components affect Zn availability. Phytate (PA) is a P storage substance found in foods such as beans, tubers and cereal grains. Ingestion of phytic acid substantially reduces Zn bioavailability due to the formation of insoluble salts (Pallauf & Rimbach, 1997). Chronic low Zn intake and high PA intake are risk factors for severe Zn deficiency. It is reported that intake of diets rich in insoluble dietary fibre impairs mineral absorption (Franz *et al.* 1980; Sandberg *et al.* 1982; Donangelo & Eggum, 1986; Jiang, 1986). However, reduction of the phytate content of bran and barley husks was found to improve Zn balance in human subjects who consumed such fibre (Navert *et al.* 1985). Many studies have shown that dietary fibre does not affect mineral balance (Bagheri & Gueguen, 1982; Mason *et al.* 1990; Sandstrom *et al.* 2000). PA present in fibre materials is involved in the impairment of mineral absorption observed upon ingestion of high-fibre diets (Thebaudin *et al.* 1997). The effects of insoluble fibre itself on Zn absorption have not been fully clarified.

Fairweather-Tait & Wright (1990) reported that sugar-beet fibre, a highly fermentable type of insoluble fibre, enhanced Zn absorption in rats. It is well known that some kinds of highly fermentable indigestible carbohydrates

Abbreviations: CE, cellulose; CF, corn husk fibre; PA, phytate; PC, pineapple core fibre; YB, yam-bean root fibre; WS, watermelon skin fibre.

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stimulate Ca or Mg absorption from the large intestine (Ohta *et al.* 1994; Hara *et al.* 1996; Younes *et al.* 1996), but the relationship between intestinal fermentation and Zn absorption is not understood. In addition, the effects of intestinal microbial phytase activity on mineral utilization remain controversial (Wise & Gilburt, 1982; Miyazawa *et al.* 1996).

In the present study, we examined the effects of ingestion of four kinds of insoluble dietary fibre from unexploited sources, with or without phytic acid, on Zn absorption in comparison with cellulose as a control. We determined whether these types of fibre are effective in alleviating Zn deficiency carrying growth retardation and anorexia caused by phytate ingestion. We also investigated the involvement of caecal fermentation in Zn absorption, and effects of feeding PA and these insoluble fibre on Fe absorption.

Experimental methods

Fibre preparations

We prepared insoluble fibre from three sources, watermelon skin, yam-bean root pulp and pineapple core, as watermelon skin fibre (WS), yam-bean root (*Pachyrhizus erosus*) fibre (YB) and pineapple core fibre (PC). The raw materials were each finely crushed with a waste disposal unit and washed with flowing water. The residue was collected and boiled in distilled water for 30 min to remove starch and water-soluble components, then washed with ethanol (950 g/l) with shaking for 10 h and left to stand overnight. The residue was then collected as insoluble fibre. To remove minerals from the fibre, each preparation was treated with 0·1 M-HCl for 1 h, washed several times by repeated centrifugation, and then washed with deionized water until the pH of the fibre suspension became constant. The deionized fibre residue was further washed with ethanol (950 g/l) and dried (in an oven at 50°C or in a freeze-drier). These fibre materials were milled to a fine powder by means of a Willey mill equipped with a 1 mm diameter pore sieve (Ikemoto Rika, Tokyo, Japan) before use in the experiments.

Table 1. Composition of test and stock diets (g/kg)*

	Phytate-free diet	Phytate diet
Powdered egg white†	135	135
Corn oil‡	45·0	45·0
Mineral mixture§	35·8	35·8
Vitamin mixture‖	9·00	9·00
Choline bitartrate	3·60	3·60
Granulated vitamin E¶	0·90	0·90
Phytic acid dodecasodium salt **		13·5
Cellulose or test fibre materials††	100	100
Sucrose	671	657

* The test diets contained 6·75 mg Zn/kg diet and the stock diet contained 35 mg Zn/kg diet as $ZnCo_3$.
† Taiyo Kagaku, Yokkaichi, Japan.
‡ Retinyl palmitate (7·66 μmol/kg diet) and ergocalciferol (0·0504 μmol/kg diet) were added to corn oil.
§ The mineral mixture was prepared as established by the AIN-76 Workshop held in 1989 (Reeves, 1989) except for Zn. It provided (mg/kg diet): Zn 6·75, Ca 4042, P 2697, K 3371, Mg 338, Fe 90·0, I 0·29, Mn 9·00, Cu 5·40, Na 3851, Cl 5889, Se 0·95, Mo 0·90, Cr 0·45, B 0·45, V 0·23, Sn 1·80, As 0·90, Si 18·0, Ni 0·90, F 2·45, Co 0·18.
‖ The vitamin mixture was prepared in accordance with the AIN-76 mixture (American Institute of Nutrition, 1977) except that D-biotin, menadione and L-ascorbic acid were added at 7·37 μmol, 5·23 μmol (American Institute of Nutrition, 1980) and 256 mmol (Harper, 1959)/kg diet respectively.
¶ Vitamin E granules (Juvela, Eisai, Tokyo, Japan) supplied 381 μmol all-*rac*-α-tocopheryl acetate/kg diet.
** 13·5 g dodecasodium salt/kg from rice (purity 94 %, loss on drying 8·3 %; Sigma Chemical, St Louis, MO, USA) in diet equals to 8·3 g phytic acid/kg.

Animals and diets

The composition of the diets is shown in Table 1. The types of fibre tested in the present study were the three described earlier, and corn husk fibre (CF) (Cellfer no. 200; Nippon Shokuhin Kako, Tokyo, Japan), and powdered cellulose (CE) (Pfizer, New York, NY, USA) as a control. These five types of fibre were individually mixed with a sucrose-based fibre-free semipurified diet (100 g/kg diet). The diet contained 6·75 mg (0·103 mmol) Zn/kg as $ZnCO_3$. We selected this dietary Zn level as the minimal concentration that does not cause growth retardation, but produces a marginal Zn deficiency, as shown in a previous study (Hara *et al.* 2000). Egg white was used as the protein source because of its low Zn content (<1 mg (0·015 mmol) Zn/kg). The Zn content and other information on the tested fibre materials are shown in Table 2. Zn derived from the fibre materials in the test diets was less than 5 % of the total amount in the diet. The corn-husk fibre is rich in hemicellulose. WS is high in CE and low in hemicellulose, and one third of the constitutive sugar is uronic acid. The constitutive sugar in YB is also rich in uronic acid. D-Biotin was added at 7·37 μmol/kg diet to prevent biotin deficiency, because egg white was used as the protein source. We prepared diets with and without phytic acid (12·6 mmol/kg) added as the sodium salt.

Male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan), 3 weeks of age, were housed individually in stainless-steel wire-bottomed cages. They were given free access to deionized water and a Zn-adequate semipurified stock diet (35 mg (0·535 mmol) Zn/kg diet) for a 1 week acclimatization period. The animals were divided into ten groups of eight rats each, based on body weight, by a

Table 2. Tested fibres information*

	CH†	WS	YB	PC
TDF (g/kg)	853	918	695	958
Lignin (g/kg)	13·2	3·1	22·2	9·3
Cellulose (g/kg)	190	711	480	621
Hemicellulose (g/kg)	641	35	146	169
Ash (g/kg)	1·65	2·03	2·43	15·20
Uronic acid (mmol/kg)‡	100	641	713	258
Zinc content (μmol/kg)	11·6	41·4	25·4	33·0

CH, corn husk fibre; WS, watermelon skin fibre; YB, yam-bean root fibre; PC, pineapple core fibre: TDF, total dietary fibre.
* TDF, ash, neutral detergent fibre and acid detergent fibre (g/kg) in the fibre materials as determined by the Association of Official Analytical Chemists (1997) method. Lignin, cellulose and hemicellulose contents were calculated from these data.
† Comercial corn husk was used; Cellfer no. 200, Nippon Shokuhin Kako, Tokyo, Japan. Powdered cellulose (Pfizer, New York, NY, USA) was used as control fibre. The TDF and zinc contents were 910 g/kg and 2·70 μmol/kg.
‡ Uronic acid contents were determined by the dimethylphenol method.

randomized block design, and the animals were given the assigned test diet containing one of the five different types of fibre materials with or without PA. They were maintained in a temperature- and humidity-controlled room (22–24°C, 40–60 %) with a 24 h light-dark cycle (dark period from 20.00 to 08.00 hours). Food intake and body weight were recorded every day. Faeces excreted during days 7–10 and days 6–19 of the test period were collected to evaluate apparent Zn and Fe absorption. On day 19, the rats were anaesthetized (50 mg pentobarbital sodium/kg body weight; Abbott, Chicago, IL, USA), and samples of aortic blood were collected. The caecum and a segment of the ileum (20–5 cm upstream from the ileocaecal valve), with their contents removed, were immediately frozen in liquid N_2 and stored at −40°C until subsequent analyses. The right femur was removed and carefully cleaned of adherent tissue.

This study was approved by the Hokkaido University Animal Committee, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

Analytical procedures

The total dietary fibre content of each of the fibre materials was measured by means of a commercial kit (TDF-100 A; Sigma, St Louis, MO, USA) based on the method of the Association of Official Analytical Chemists (1997).

The Zn and Fe concentrations in the test diets, faeces, femur, caecal contents and plasma and the caecal pH were determined by the previously described methods (Hara *et al.* 2000). Freeze-dried faeces were well milled. The freeze-dried whole femur and aliquots of the test diets and powdered faeces were wet-ashed and Zn and Fe (faeces only) concentrations were measured by atomic absorption spectrometry (AA-6400F; Shimadzu, Kyoto, Japan) after suitable dilution. The recovery of Zn in diet was 99·7 %. The Zn concentration in plasma was measured by means of a commercially available kit (Zn test-Wako; Wako Pure Chemical Industries, Osaka, Japan).

The caecal weight, including that of the caecal contents, was measured, and the caecal wall weight was measured again after collecting the contents and washing out the caecum with saline. The collected caecal contents were homogenized with four volumes of deionized water and the pH was measured as the caecal pH and the Zn content was measured after wet-ashing as the total Zn pool of the caecum. The supernatant fraction obtained upon centrifugation of the caecal homogenate (30 000 ***g*** at 4°C for 20 min) was analysed after deproteinization by treatment with 0·5 M-perchloric acid to determine the soluble Zn concentration in the caecal contents. The Zn concentrations in the wet-ashed caecal homogenate and the deproteinized supernatant fraction were measured by atomic absorption spectrometry as described earlier. The concentrations of short-chain fatty acids and other organic acids (acetic, propionic, butyric, valeric, succinic and lactic acid) in the supernatant fractions of the caecal contents were measured by ion-exclusion chromatography using an HPLC system equipped with a solvent delivery system (SLC-10AVP; Shimadzu), a double ion-exchange column (Shim-pack SCR-102H, 8 × 300 mm; Shimadzu) and an electroconductivity detector (CDD-6A; Shimadzu). Crotonic acid was added to the supernatant fraction of the caecal contents as an internal standard, the mixture was treated with NaOH, centrifuged (13 000 ***g*** at 4°C for 20 min), and impurities were removed by extraction with hexane before HPLC analysis (Hoshi *et al.* 1994). The mobile phase consisted of 5 mmol *p*-toluenesulfonic acid/l.

The ileal contents were collected by washing out the lumen of the ileal segment with saline, and the chyme washed out was pooled for every group. Each specimen was washed with deionized water several times with vigorous shaking and then washed with absolute ethanol. The settling volume (Takeda & Kiriyama, 1979) of the fibre materials tested and that of the lyophilized residue of the ileal contents were measured. Briefly, these samples were macerated in water, degassed and transferred to a 100 ml volumetric cylinder to measure the volume of the residue which had settled after 24 h.

Calculations and statistical analyses

Apparent Zn absorption was calculated as follows:

$$\text{apparent Zn (Fe) absorption (\%)} = 100 \times (\text{Zn (Fe) intake} - \text{faecal Zn (Fe) excretion})/\text{Zn (Fe) intake}.$$

The weight of the caecal contents was determined by subtraction of the caecal wall weight from the total caecal weight. We evaluated the faecal dry weight:ingested fibre weight during the balance period as relative faecal weight. The effects of the dietary fibre and phytate were analysed by two-way ANOVA. Duncan's multiple range test was used to determine whether mean values were significantly different (Duncan, 1995; $P<0{\cdot}05$). These statistical analyses were performed by the General Linear Models procedure of SAS (SAS version 6.07, Statistical Analysis Systems Inc., Cary, NC, USA).

Results

Effects of the phytate and fibres on growth

Insoluble fibre in the diets without PA did not affect body-weight gain or food intake (Table 3). Body-weight gain in rats fed CH, WS or YB with PA (CH/PA, WS/PA, YB/PA groups) were higher (33, 77 and 79 % respectively, in each instance) than in rats fed the CE diet with added PA (CE/PA). Food intakes in these groups were also higher than the CE/PA group (14, 24 and 25 % respectively, in each instance). Food efficiency ratio in rats fed tested fibre with PA were higher than that of the CE/PA group. Feeding of PA influenced body-weight gain as indicated by the results of two-way ANOVA. In the CE-fed control groups, body-weight gain in rats fed PA was less than half of that in rats fed the PA-free diet. However, body-weight gain in both the WS/PA and YB/PA groups was 70 % of that in the corresponding groups without PA (WS, YB).

Table 3. Food intake, body-weight gain and food efficiency ratio of rats fed diets containing different fibre materials for 19 d*

(Mean values for eight rats per group)

	Food intake (g/d)	Body-weight gain (g)†	Food efficiency ratio (g/g)
Without PA			
CE	16·8[a]	133[a]	0·417[a]
CH	16·9[a]	134[a]	0·418[a]
WS	17·7[a]	142[a]	0·424[a]
YB	17·8[a]	142[a]	0·422[a]
PC	18·1[a]	143[a]	0·416[a]
With PA			
CE/PA	11·5[c]	57[d]	0·260[d]
CH/PA	13·1[b]	76[c]	0·303[c]
WS/PA	14·3[b]	101[b]	0·370[b]
YB/PA	14·4[b]	102[b]	0·375[b]
PC/PA	11·7[c]	65[cd]	0·291[c]
Pooled SEM	0·43	4·5	0·0099
ANOVA (*P* values):			
F	<0·001	<0·001	<0·001
PA	<0·001	<0·001	<0·001
F×PA	0·002	<0·001	<0·001

PA, phytate; CE, cellulose; CH, corn husk fibre; WS, watermelon skin fibre; YB, yam-bean root fibre; PC, pineapple core fibre; F, fibre.
[a,b,c,d]Mean values within a column with unlike superscript letter were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 444.
† The overall average initial body weight was 91 g.

Effects of phytate and fibres on zinc and iron

In rats fed the PA-free diets, the rate of Zn absorption in the YB group was 13 % higher than that in the CE group during days 7–10. The rate of Zn absorption during days 16–19 was not different among the five groups fed diets without PA (Table 4). In the CE-fed control groups, the rate in the rats fed the CE/PA diet was 46 % lower in days 7–10 and 70 % lower in days 16–19 compared with that in the rats fed CE. The rate of Zn absorption in rats fed the WS/PA diet was 38 and 140 % higher than in rats fed the CE/PA diet in days 7–10 and days 16–19 respectively. Those in the YB/PA and PC/PA groups were similar to that in the CE/PA group in days 7–10, but 80 and 50 % higher in days 16–19 respectively. The rates of Zn absorption in days 16–19 in rats fed the CE/PA and CH/PA diets was 40 % less compared with that in days 7–10. In contrast, those in the WS/PA and YB/PA groups did not change comparing the values for the two periods. The difference in the rate of Zn absorption between CE/PA and WS/PA became larger in days 16–19 than that in days 7–10. Changes in the amount of Zn absorption as a result of feeding the insoluble fibre and PA tended to be similar to those observed in the rate of apparent Zn absorption, except for several significant differences due to expanding differences between groups. The amount of Zn absorption in rats fed the WS/PA and YB/PA diets was 107 and 55 % higher respectively than those fed the CE/PA even in days 7–10.

The Fe absorption ratio was not influenced by PA ingestion in both days 7–10 and days 16–19 (Table 4). The Fe absorption ratio in days 7–10 was not influenced by both fibre and PA ingestion as a result of ANOVA. Fe absorption in days 16–19 was higher in the CH, WS and YB groups than that in the CE group in rats fed PA-free diets.

Femoral and serum zinc concentration

The femoral Zn concentration in the WS group without PA was higher than that in the CE group without PA. In the PA-fed rats, femoral Zn concentrations were approximately 50 % lower than those in rats fed the PA-free diets (Table 5). Comparing the five groups of rats fed the PA-containing diets, the Zn concentration in the femur was higher in the

Table 4. Respective effects of dietary fibre and phytate on zinc and iron absorption in rats (% intake)*

(Mean values for eight rats per group)

	Zinc absorption		Iron absorption	
	Day 7–10	Day 16–19	Day 7–10	Day 16–19
Without PA				
CE	74·1[b]	80·0[a]	39·3	19·1[c]
CH	79·8[ab]	82·3[a]	52·6	34·5[ab]
WS	81·3[ab]	83·9[a]	40·0	34·1[ab]
YB	83·8[a]	82·4[a]	44·0	32·3[ab]
PC	79·7[ab]	83·9[a]	41·1	28·1[bc]
With PA				
CE/PA	40·5[d]	24·0[d]	45·0	22·8[bc]
CH/PA	44·7[d]	26·9[d]	44·3	30·5[bc]
WS/PA	55·9[c]	57·6[b]	39·2	42·8[a]
YB/PA	46·8[d]	43·3[c]	45·7	33·9[ab]
PC/PA	43·7[d]	36·9[c]	44·2	26·3[bc]
Pooled SEM	2·04	2·79	4·69	3·68
ANOVA (*P* values)				
F	<0·001	<0·001	0·413	<0·001
PA	<0·001	<0·001	0·927	0·486
F×PA	0·779	<0·001	0·629	0·464

PA, phytate; CE, cellulose; CH, corn husk fibre; WS, watermelon skin fibre; YB, yam-bean root fibre; PC, pineapple core fibre; F, fibre.
[a,b,c,d]Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 444.

Table 5. Femoral and plasma zinc concentration in rats fed diets containing different fibre materials for 19 d*

(Mean values for eight rats per group)

	Femoral zinc concentration (μmol/g DM)	Plasma zinc concentration (μmol/l)
Without PA		
CE	2·09[b]	13·5[b]
CH	2·24[ab]	15·7[ab]
WS	2·35[a]	17·0[a]
YB	2·11[b]	15·2[ab]
PC	2·23[ab]	15·1[ab]
With PA		
CE/PA	1·00[d]	5·11[c]
CH/PA	1·10[cd]	7·04[c]
WS/PA	1·15[cd]	5·56[c]
YB/PA	1·22[c]	5·81[c]
PC/PA	1·16[cd]	4·36[c]
Pooled SEM	0·0602	0·905
ANOVA (*P* values)		
F	0·018	0·094
PA	<0·001	<0·001
F×PA	0·146	0·381

PA, phytate; CE, cellulose; CH, corn husk fibre; WS, watermelon skin fibre; YB, Yam-bean root fibre; PC, pineapple core fibre; F, fibre.
[a,b,c,d]Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 444.

WS, YB and PC groups than in the CE group. PA ingestion severely decreased plasma Zn concentration, however, fibre did not affect the results of two-way ANOVA.

Caecal variables and caecal fermentability of the dietary fibre

The WS and PC groups had a larger caecal wall than that in the CE groups, with or without PA (Table 6). The changes in weight of the caecal contents were similar to those in caecal-wall weight. In rats fed the PA-free diets, the pH of the caecal contents was higher in the PC group than in the CH or WS groups. In the PA-fed rats, the pH of the caecal contents in the WS group was the lowest, and the pH of the WS and YB groups was lower than that of the other fibre groups. In the WS and YB groups, the pH of the caecal contents in the rats fed the PA-containing diet was lower than that in the case of the rats fed the PA-free diet. In the other fibre groups, the pH of the PA-fed groups was identical to that in the case of the groups fed the PA-free diet. The caecal pH in all groups fed PA-free diets was slightly alkaline.

Table 6. Caecal wall weight and caecal contents weight and caecal pH in rats fed diets containing different fibre materials for 19 d*

(Mean values for eight rats per group)

		Caecal contents	
	Wall wet weight (g)	Wet weight (g)	pH
Without PA			
CE	0·522[ef]	2·52[cd]	7·36[ab]
CH	0·582[cde]	3·05[bc]	7·23[bc]
WS	0·630[abc]	3·48[ab]	7·26[bc]
YB	0·586[cde]	3·25[abc]	7·37[ab]
PC	0·700[a]	3·70[ab]	7·55[a]
With PA			
CE/PA	0·452[f]	1·93[d]	7·54[a]
CH/PA	0·465[f]	2·48[cd]	7·36[ab]
WS/PA	0·685[ab]	3·85[a]	6·80[d]
YB/PA	0·530[def]	3·26[abc]	7·09[c]
PC/PA	0·610[bcd]	3·23[abc]	7·53[a]
Pooled SEM	0·029	0·024	0·074
ANOVA (*P* values)			
F	<0·001	<0·001	<0·001
PA	0·004	0·102	0·062
F×PA	0·043	0·194	<0·001

PA, phytate; CE, cellulose; CH, corn husk fibre; WS, watermelon skin fibre; WB, yam-bean root fibre; PC, pineapple core fibre; F, fibre.
[a,b,c,d,e,f]Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 444.

Organic acid concentrations in the caecal contents are shown in Table 7. Total short-chain fatty acid concentrations were not different among the five fibre groups fed PA-free diets. In rats fed the PA-free diets, only the acetate concentration in the WS group was higher than that in the CE group. In rats fed the PA-containing diets, the concentrations of total and all three major short chain fatty acids were higher in the WS and YB groups than in the CE group, except for propionate in the YB group. As indicated by the results of two-way ANOVA, in the case of most caecal organic acids, there were significant interactions between fibre and PA.

Relative values of faecal dry weight in relation to ingested fibre weight are shown in Table 8. The relative values of faecal excretion in the YB group were the lowest among the five fibre groups in the case of rats fed PA-free diets, and the value for the WS group was the lowest among rats fed the PA-containing diets in both periods, days 7–10 and days 16–19. In both fibre groups, the values were lower than those in the CE group in the case of rats fed the diet with or without PA. The relative faecal weight in the WS and PC groups with PA was lower than that in the

Table 7. Concentrations of short chain fatty acids (SCFA) and other organic acids (μmmol/g contents) in the caecal contents of rats fed diets containing different fibre materials for 19 d*

(Mean values for eight rats per group)

	Acetate	Propionate	Butyrate	Total SCFA†	Succinate	Lactate
Without PA						
CE	42·5^{cd}	10·6^{b}	12·8^{abc}	68·5^{cde}	0·785^{ab}	0·759
CH	40·8^{cd}	11·1^{b}	15·9^{a}	70·2^{bcd}	0·777^{ab}	1·31
WS	57·2^{ab}	9·47^{b}	10·4^{bcd}	79·1^{bc}	0·228^{b}	0·336
YB	51·9^{bc}	10·8^{b}	10·4^{bcd}	75·4^{bcd}	0·722^{ab}	2·09
PC	42·3^{cd}	9·54^{b}	6·64de	59·6^{def}	2·79^{ab}	0·486
With PA						
CE/PA	33·5^{d}	9·43^{b}	5·95^{e}	51·1^{f}	0·793^{ab}	0·414
CH/PA	31·7^{d}	9·45^{b}	9·31^{cde}	52·8^{ef}	0·424^{b}	0·625
WS/PA	65·6^{ab}	13·9^{a}	15·9^{a}	97·5^{a}	3·31^{a}	3·48
YB/PA	58·4^{ab}	12·3^{ab}	13·8^{ab}	86·7^{ab}	0·512^{b}	0·936
PC/PA	42·2^{cd}	10·2^{b}	7·27de	61·3^{def}	0·449^{b}	1·91
Pooled SEM	4·26	0·86	1·43	5·63	0·794	0·922
ANOVA (*P* values)						
F	<0·001	0·105	<0·001	<0·001	0·397	0·663
PA	0·809	0·167	0·391	0·856	0·941	0·418
F×PA	0·123	0·006	<0·001	0·004	0·024	0·126

PA, phytate; CE, cellulose; CH, corn husk fibre; WS, watermelon skin fibre; YB, yam-bean root fibre; PC, pineapple core fibre; SCFA, short-chain fatty acid.
[a,b,c,d,e,f]Mean values with unlike superscript letters were significantly different (P<0·05).
* For details of diets and procedures, see Table 1 and p. 444.
† Total SCFA is sum of acetic, propionic, butyric, isobutyric, valeric and isovaleric acids.

corresponding fibre groups without PA in days 7–10, and the difference between rats fed diets with and without PA was significant in the case of the WS groups in days 16–19.

Caecal zinc pool and concentration

There was no difference in the caecal total Zn pool or soluble Zn pool among the groups fed the PA-free diets, but the concentrations of soluble Zn in the WS and PC groups were lower than that in the CE group (Table 9). In the rats fed the PA-containing diets, the total and soluble Zn pools, but not the soluble Zn concentration, in the WS group were higher than those in the CE group. The soluble Zn concentration in the PC group was lower than that in the other PA groups. In rats fed WS or YB, higher values for all caecal Zn variables were obtained in the case of the groups fed the PA-containing diets than in the case of the groups

Table 8. Relative values of fecal dry weight in relation to ingested fibre weight during the balance periods (days 7–10 and days 16–19)*

(Mean values for eight rats per group)

	Days 7–10 (g/g)	Days 16–19 (g/g)
Without PA		
CE	1·08^{ab}	1·04^{a}
CH	1·02^{bcd}	1·01 ^{ab}
WS	0·928^{d}	0·874^{c}
YB	0·793^{e}	0·724^{d}
PC	1·18^{a}	0·985^{abc}
With PA		
CE/PA	1·04^{bc}	1·11^{a}
CH/PA	1·05^{bc}	1·09^{a}
WS/PA	0·697^{e}	0·510^{e}
YB/PA	0·719^{e}	0·674^{d}
PC/PA	0·966^{cd}	0·894^{bc}
Pooled SEM	0·037	0·043
ANOVA (*P* values)		
F	<0·001	<0·001
PA	<0·001	0·012
F×PA	0·002	<0·001

PA, phytate; CE, cellulose; CH, corn husk fibre; WS, watermelon skin fibre; YB, yam-bean root fibre; PC, pineapple core fibre; F, fibre.
[a,b,c]Mean values within a column with unlike superscript letters were significantly different P<0·05.
* For details of diets and procedures, see Table 1 and p. 444.

Table 9. Caecal total zinc pool, soluble zinc pool and soluble zinc concentration*

(Mean values for eight rats per group)

	Total zinc pool (μmol)	Soluble zinc	
		Pool (μmol)	Concentration (μmol/g contents)
Without PA			
CE	0·389^{cd}	0·0514^{c}	0·0205^{ab}
CH	0·420^{cd}	0·0625^{bc}	0·0208^{ab}
WS	0·341^{d}	0·0446^{c}	0·0129^{c}
YB	0·330^{d}	0·0501^{c}	0·0155^{bc}
PC	0·459^{bcd}	0·0434^{c}	0·0116^{c}
With PA			
CE/PA	0·532^{bcd}	0·0425^{c}	0·0225^{a}
CH/PA	0·648^{ab}	0·0541^{bc}	0·0241^{a}
WS/PA	0·766^{a}	0·0908^{a}	0·0236^{a}
YB/PA	0·582^{abc}	0·0732^{ab}	0·0224^{a}
PC/PA	0·349^{d}	0·0467^{c}	0·0146^{c}
Pooled SEM	0·069	0·0064	0·0018
ANOVA (*P* values)			
F	0·186	<0·001	0·002
PH	<0·001	<0·001	0·008
F×PA	0·005	0·121	<0·001

PA, phytate; CE, cellulose; CH, corn husk fibre; WS, watermelon skin fibre; YB, yam-bean root fibre; PC, pineapple core fibre; F, fibre.
[a,b,c,d]Mean values with unlike superscript letters were significantly different (P<0·05).
* For details of diets and procedures, see Table 1 and p. 444.

Table 10. Settling volume (ml/g) of fibres and ileal contents in water*

	Fibre	Ileal contents
Cellulose	4·9	6·1
Corn-husk fibre	4·7	6·5
Watermelon skin fibre	12	44
Yam-bean root fibre	10	44
Pineapple core fibre	16	64

* For details of preparation of fibres and procedures, see p. 444.

fed the PA-free diets. Ingested fibre sources did not affect Zn solubility (P=0·298 for dietary fibre (F), P=0·036 for PA, P=0·755 for F × PA; overall values were 15·6 (SE 1·82) % in PA-free group, 11·5 (SE 0·789) % in PA-fed groups).

Settling volume of each type of fibre and ileal contents

Before ingestion, the settling volume of CE and that of CH was about 5 ml/g (Table 10). The values for WS, YB and PC were 2- to 3·3-fold higher than those for CE. The ileal chyme residue showed a much greater settling volume than the fibre itself. In particular, values for ileal chyme in the case of the rats fed the WS, YB or PC were about 4-fold higher than the values for the original fibre materials.

Discussion

In the present study, PA ingestion caused markedly lowered Zn absorption with depressed food intake, body-weight gain in the CE-fed control groups, which agrees with the results of a previous report (Zhou *et al.* 1992). We demonstrated that the depressed growth and Zn absorption by PA recovered considerably as a result of ingestion of WS and YB (Table 3). We observed a significant correlation between the Zn absorption ratio and body-weight gain or the food efficiency ratio in rats fed PA-containing diets (R 0·571, P<0·001, n 40).

We did not adopt a pair-feeding design in this experiment because we measured changes in food intake and growth as an important indicator of Zn deficiency. In man severe Zn deficiency results in growth retardation (Ferguson & Gibson, 1993). It is important and more reliable to cause these symptoms than only to observe increasing Zn absorption in respect of today's nutritional problem. We demonstrated that feeding WS or YB improved not only body-weight gain, but also food efficiency depressed by PA ingestion. These results strongly suggest that these insoluble fibres increased Zn absorption, then recovered depressed growth caused by PA. We showed that ingestion of PA induced severe reduction of plasma Zn concentration indicating severe Zn deficiency (Table 3). Zn deficiency induces anorexia and results in growth retardation (MacDonald, 2000). The increase in the amount of Zn absorbed as a result of feeding the insoluble fibres may effectively supply Zn to the tissues. Increase in Zn supply may partially relieve anorexia and restore growth, however, not sufficiently to increase plasma Zn concentration.

Zn absorption in rats fed PA with the WS or YB was maintained at a constant level throughout the experimental period, although the absorption in the rats fed PA with CE decreased later in the test period. Our previous study showed that the Zn absorption ratio was lowered in rats fed a severely Zn-deficient diet (1 mg Zn/kg diet) (Hara *et al.* 2000). Severe Zn deficiency in the mucosal cells may be involved in the reduction of the rate of Zn absorption observed as a result of PA feeding in the CE group. In rats fed the WS or YB with PA, the amount of Zn absorbed may be sufficient to maintain Zn absorption capacity in the intestine.

Fe is another nutrient which interacts with PA, however, dietary PA did not affect Fe absorption in the present study. One reason for this may be that we used ferric citrate as the Fe source. In most papers suggesting that PA has an inhibitory effect on Fe absorption, inorganic salt was used as an Fe source (Larsson *et al.* 1996; Tidehag *et al.* 1996; Sandberg *et al.* 1999).

It is known that promotion of intestinal fermentation is a mechanism for stimulatory effects of dietary fibre on mineral absorption. Ingestion of highly fermentable fibre stimulates Ca absorption as described earlier (Hara *et al.* 2000). Several mechanisms are proposed to be responsible for the stimulatory effect on Ca absorption; expansion of the lumen surface due to enlargement of the caecum, an increase in soluble Ca concentration in the large intestine contents as a result of a decrease in lumen pH and stimulation of Ca absorption by acetate and propionate (Trinidad *et al.* 1996; Hara *et al.* 1999.) On the first mechanism, the caecal wall weight in the present study was 1·2 or 1·3 times greater in the WS or PC group than in the CE group (Table 6). This difference is probably too small to show a significant effect in promotion of mineral absorption. The caecal wall weight has been found to increase by 1·6-fold or more as a result of feeding types of fibre that are effective in enhancing mineral absorption (Lopez *et al.* 1998; Hara *et al.* 1999). On the second mechanism, soluble Zn concentrations in the caecal contents did not increase as the caecal pH decreased (Tables 6 and 9). Phytase in the caecum possibly acts to solubilize the Zn and may partly contribute to the Zn absorption-promoting effects of these types of fibre. However, the concentration and also the solubility of Zn in the caecum did not increase as a result of feeding the types of fibre tested, as described earlier (Table 9). On the last mechanism, acetate but not propionate was somewhat higher in the groups fed the effective types of fibre than those in the group fed the CE-containing diet with added PA (Table 7). These results suggest that caecal fermentation is partly involved, but not completely, in acceleration of Zn absorption in the case of feeding the types of insoluble fibre tested. In addition, ingestion of PC also enhanced Zn absorption lowered by PA without any increases in caecal short-chain fatty acids (Table 7). The result indicates that the PC is a poorly fermentable fibre, and reveals that feeding PC increases Zn absorption in a manner independent of caecal fermentation.

Feeding PA increased the total short-chain fatty acid concentration in the caecal contents and lowered the relative faecal excretion in rats fed WS or YB. PA feeding may facilitate caecal fermentation of these types of insoluble fibre. Inositols, degradation products of PA, may be used by

some bacteria in the large intestine. It is possible that fermentation of WS and YB is enhanced by bacterial species activated by PA.

A common property shared by WS, YB and PC is the capacity to cause a large increase in settling volume (Table 10). The bulking effect of dietary fibre slows the transit of chyme through the small intestine (Johansen, 1994; Lin, 1997), which might lead to an increase in Zn absorption. However, the settling volume of the ileal contents of rats fed PC was the highest among the types of fibre tested, as shown in Table 10, and the extent of improvement of Zn absorption in the PC group was lower than that in the WS group. It is possible that the bulking effect of PC it too high for severely Zn-deficient rats.

The chelating properties of the insoluble fibre are possibly involved in the mechanism of enhancement of Zn absorption by the fibre. We showed high uronic acid content in WS and YB (Table 2). The uronic acid fibres possibly have the property for binding metals. Further studies must be done to elucidate the mechanism of action of the insoluble dietary fibre in promoting Zn absorption.

In conclusion, WS, YB or PC significantly restored Zn absorption reduced in rats fed a low-Zn diet with added PA. In particular, WS and YB enhanced Zn absorption strongly enough to ameliorate growth retardation and anorexia caused by severe Zn deficiency. Our findings suggest that caecal fermentation is a possible, but not a sole, factor involved in these effects.

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